



Original Article

Adaptive immunoregulation of luteolin and chlorogenic acid in lipopolysaccharide-induced interleukin-10 expression

Chu-Yen Cheng^a, Chia-Chou Yeh^{a,b*}

^aDepartment of Chinese Medicine, Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Chia-Yi, Taiwan, ^bSchool of Post-Baccalaureate Chinese Medicine, Tzu Chi University, Hualien, Taiwan

Submission : 23-Jan-2019
Revision : 01-Mar-2019
Acceptance : 11-Apr-2019
Web Publication : 24-Jun-2019

ABSTRACT

Objective: To investigate the mechanism of the adaptive effect of two compounds in *Lonicerae japonica* flos (LJF), luteolin (LUT) and chlorogenic acid (CGA), on the expression of interleukin (IL) IL-10 and IL-6. **Materials and Methods:** RAW264.7 cells receiving lipopolysaccharide (LPS) were pretreated with CGA and LJF. The expression of pro-inflammatory cytokines and IL-10 was evaluated by reverse transcription-polymerase chain reaction. Moreover, the concentrations of IL-10 and IL-6 were measured by enzyme-linked immunosorbent assay in the culture medium obtained 24 h after LPS treatment. Nuclear extracts of RAW264.7 cells, pretreated with CGA or LUT and LPS, were prepared after 6 h, and C/EBP β and C/EBP δ were measured by Western blotting. Nuclear factor- κ B (NF- κ B) activity was measured by electrophoretic mobility shift assay. The phosphorylated form of I κ B, ERK1/2, p38, JNK, and I κ B, ERK2, p38, or JNK were also measured by Western blotting. **Results:** CGA enhanced the LPS-induced expression of IL-10 and IL-6, and increased NF- κ B, Sp1, C/EBP β and δ . The effect of CGA is interfered with Lut by suppressing the phosphorylation of I κ B and p38, and NF- κ B activity. In the event, IL-6 was suppressed and IL-10 was not influenced. **Conclusion:** LUT and CGA, which are abundant in LJF that is one of the ingredients in Gingyo-san, have adaptive immunoregulative effect on the expression of IL-10.

KEYWORDS: Chlorogenic acid, Interleukin-10, *Lonicerae japonica* flos, Luteolin

INTRODUCTION

Interleukin (IL) (IL-10) functions as a cytokine synthesis-inhibiting factor and plays a role in the activity inhibition of Th1 cells [1]. IL-10 has various biological effects on different cell types. In macrophages, IL-10 suppresses ligand-induced activation and production of pro-inflammatory cytokines from macrophages [2,3]. IL-10 inhibits the proliferation as well as cytokine synthesis of CD4⁺ T cells [4]. Other immunosuppressive effects on eosinophils, neutrophils, and dendritic cells have also been well documented [5]. IL-10 on the other hand has been shown to have an immunostimulation effect on cytotoxic T cells [6] and can act as a growth co-stimulator for thymocytes and mast cells [7]. The immunosuppressive functions of IL-10 are an important mechanism for protecting the host from the harmful effects of exaggerated inflammatory and immune responses under the situation of microbial infection [2,3]. Several reports suggested that lipopolysaccharide (LPS)-induced IL-10 expression was mediated through the pathway of nuclear factor- κ B (NF- κ B)- and mitogen-activated protein kinase (MAPK)-induced protein expression and DNA binding of C/EBP δ [8-10].

Luteolin (LUT), a flavone found in high concentrations in celery, green pepper, perilla leaf and seeds, and *Lonicerae japonica* flos (LJF) [11], is one of the most potent and efficacious flavonoid inhibitors of LPS-induced tumor necrosis factor- α (TNF- α) and IL-6 production and inducible nitric oxide synthase (iNOS) expression [12,13]. LUT blocks LPS-induced NF- κ B and AP-1 signaling and pro-inflammatory gene expression [13,14]. Chlorogenic acid (CGA), an ester of caffeic acid, is found with quinic acid in a wide range of fruits and vegetables and is particularly abundant in coffee [15] and *Lonicerae Flos* [16]. CGA possess antioxidant properties *in vivo* [17,18]. CGA has been reported to prevent different cancers and cardiovascular diseases in several experimental animal models [19-21]. CGA also possess chemopreventive effect through the suppression of NF- κ B, AP-1, and MAPK activation [22]. It has been reported that CGA


*Address for correspondence:

Dr. Chia-Chou Yeh,
Department of Chinese Medicine, Buddhist Dalin Tzu Chi General Hospital, 2, Min-Sheng Road, Dalin, Chiayi, Taiwan.
E-mail: yehcc0530@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Cheng CY, Yeh CC. Adaptive immunoregulation of luteolin and chlorogenic acid in lipopolysaccharide-induced interleukin-10 expression. *Tzu Chi Med J* 2020;32(2):186-92.

Access this article online	
Quick Response Code: 	Website: www.tcmjmed.com
	DOI: 10.4103/tcmj.tcmj_23_19

augmented the inducible cytokine messages, i.e., IL-10, IL-13, interferon- γ (IFN- γ), IL-6, and TNF- α that involve in the immunoregulation processes [23].

Modulation of innate immunity by natural plant products may represent an attractive strategy to prevent lung inflammation associated with dysregulated innate immune responses. In our previous study, Gingyo-san (GGS) is a powder preparation of traditional Chinese medicine and *Lonicerae Japonica* Flos is one of its ingredients; it reduced the LPS-induced lung inflammation with the effects of down-regulating inflammatory cytokines (TNF α , IL-1 β , IL-6, and IL-8) and anti-inflammatory cytokines (IL-12 and IL-4), and up-regulating IL-10 *in vivo* [24]. In this study, we tried to investigate the mechanism that the adaptive effect of two compounds in LJF, LUT and CGA, on the expression of IL-10 and IL-6.

MATERIALS AND METHODS

CGA (CAS number 327-97-9), LUT (CAS number 491-70-3) and LPS were purchased from Sigma-Aldrich Inc. (Darmstadt, Germany) DMEM medium was purchased from Invitrogen (Waltham, Massachusetts, USA). Fetal bovine serum, gentamicin, and L-glutamine were purchased from Invitrogen. All antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). SP-1, C/EBP β , C/EBP δ , and enzyme-linked immunosorbent assay (ELISA) kits of IL-10 and IL-6 were obtained from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA.

Preparation of the extract of gingyo-san and *Lonicerae japonica* flos

The medicinal plants used to prepare GGS were provided by Koda Pharmaceuticals Ltd., Taoyuan, Taiwan. Gingyo-san is commercially available in Taiwan and Japan. The extraction procedure and its components were reported [24]. LJF was prepared by the same procedure. Brief procedure: Raw herbs of LJF were soaked and rinsed in running water repeatedly to wash any dirt or impurities off. After drying, slicing, and precise scaling, Koda Pharmaceuticals attains optimal extraction via cyclic-style extraction under controlled temperature, pressure, water, and duration using fully automated and computerized equipment under vacuum and low-temperature conditions. After extraction, the residue and extract were separated by filtration, to remove dregs from the extracts. Low-temperature vacuum system is used to remove part of the decoction liquid, and then concentrate the filtered decoction liquid into herbal liquid extracts. These extracts of GGS and LJF were dissolved in pyrogen-free isotonic saline (YF Chemical, Taipei, Taiwan) and filtered through a 0.2-mm filter (Microgen, Laguna Hills, CA, USA) before use.

High-performance liquid chromatography analysis

Samples (0.5 g) of the herbal mixtures were extracted at three separate times during boiling and were mixed with 10 ml of methanol in a rotating cultivator (200 rpm, 60 min). The extracted liquid was filtered, and the final volume was adjusted to 10 ml with methanol. After passing the filtrate through a 0.45- μ m membrane filter, the samples were subjected to analysis by high-performance liquid chromatography (HPLC). Koda Pharmaceuticals Ltd. offered this HPLC. The chromatographic

system consisted of a Hitachi D-7000 Interface, L-7100 pump, L-7455 DAD, L-7200 Autosampler, Mightysil RP-18, and a GP 250 mm \times 4.6 mm (5 μ m) column. Methanol was used as the mobile phase, and components were detected at 330 nm. The injection volume was 10 μ L, and the flow rate was 0.8 mL/min. A standard solution was prepared by dissolving 1.0 mg CGA and 1.0 mg LUT 10 mL of 70% methanol. The solution was injected into the HPLC system after passage through a 0.45- μ m membrane filter [25].

Measurement of tumor necrosis factor- α , interleukin-1 β , interleukin-6, inducible nitric oxide synthase, and interleukin-10 mRNA by reverse transcription-polymerase chain reaction

Reverse transcription-polymerase chain reaction (RT-PCR) was conducted to determine the expression of mRNA in RAW264.7 cells. Cells were collected at 24 h following various treatments. Total RNA was isolated from all RAW264.7 cell preparations by the TRIzol method (Invitrogen) and according to the manufacturer's instructions. The sequences for the TNF- α , IL-1 β , IL-6, IL-10, iNOS, and β -actin PCR primers were as follows: TNF- α (antisense: 5'-AGAAGAGGCACTCCCCAAAA-3'; sense: 5'-CCGAAGTTCAGTAGACAGAAGAGCG-3'); IL-1 β (5'-AAGCTCTCACCTCAATGGA-3' and 5'-TGCCTTGAGAGGTGCTGATGT-3'); IL-10 (sense: 5'-CGTCGGATCCGCCATGCCTGGCTC ACCACTGCT-3' and antisense: 5'-CGTCTCTAGATTAGCTTTTCATTTTGATCA-3'); IL-6 (sense: 5'-GACAACTTTGGCATTGTGG -3' and antisense: 5'-ATGCAGGGATGATGTTCTG -3'); iNOS (sense: 5'-ACCAACTGACGGGAGATGAG-3', anisense: 5'-ATAGCGGATGAGCTGAGCAT-3'); and β -actin (sense: 5'-CCTAAGGCCAACCGTGAAAAG-3' and antisense: (5'-TCTTCATGGTGCTAGGAGCCA-3').

PCR reaction was performed under an annealing temperature of 61°C for TNF- α and IL-1 β , 65°C for iNOS and IL-6, and 60°C for β -actin. The intensities of gel bands were calculated with a software Gel-Pro analyzer (Meyer Instruments).

Western blot analysis

RAW264.7 cells were treated with LPS and CGA and/or LUT s and lysed with 250- μ L sample buffer (62.5 mM Tris-HCl, 2% sodium dodecyl sulfate (SDS), 10% glycerol, 50 mM dithiothreitol, 0.1% bromophenol blue, pH 6.8). Nuclear extracts were also prepared from the same samples. The protein concentration was determined with a BCA protein assay kit (Pierce, Rockford, IL, USA). Proteins (10 μ g) were separated by 10% SDS-polyacrylamide gel electrophoresis, and protein bands were electrophoretically transferred to nitrocellulose membranes. Membranes were probed with polyclonal antibodies against I κ B, JNK, p38, and ERKs (in cytosol), and SP-1, C/EBP β , and C/EBP δ (in nuclear extracts). Bound antibodies were detected with peroxidase-conjugated anti-rabbit antibodies followed by chemiluminescence (ECL system, Amersham, Buckinghamshire, UK) and autoradiographic exposure. The intensities of dark bands were calculated with a Gel-Pro analyzer [25].

Electrophoretic mobility shift assay

The nuclear extracts were prepared from the same RAW264.7 cells used for electrophoretic mobility shift assay

(EMSA) [26] with a biotin-labeled NF- κ B consensus sequence probe (Promega, Madison, WI, USA). After nuclear extraction, NF- κ B DNA-binding capacity was determined using an NF- κ B EMSA kit (Panomics Inc. Fremont, CA, USA) according to the manufacturer's instructions. Briefly, the nuclear extracts (10 μ g) were mixed with a biotin-labeled NF- κ B probe and incubated at 37°C for 30 min. DNA protein complexes were separated in a 6% nondenaturing polyacrylamide gel in a $\times 0.5$ tris-borate-EDTA (TBE) buffer at 120 V for 60 min and then transferred to a Biotodyne B nylon membrane (Pierce, Rockford, IL, USA) at 300 mA for 40 min. The membrane was fixed using an ultraviolet crosslinker, blocked with a blocking buffer, and incubated with a streptavidin-horseradish peroxidase mixture for 15 min at room temperature. After incubation, the membrane was washed and detected using an imaging system (Princeton Instruments, NJ, USA).

Statistical analysis

A one-way ANOVA was used to determine if the means were statistically significantly different ($P < 0.05$). If means were significantly different, a Tukey-Kramer *post hoc* multiple group comparison test was used to compare individual groups. Error bars in figures represent \pm standard error of mean.

RESULTS

Effect of the herbs on lipopolysaccharide-induced interleukin-10 expression

In our previous study, GGS was demonstrated to upregulate LPS-induced IL-10 [24]. The GGS is a mixture of ten crude plant ingredients. We tried to investigate the effect of GGS and ten crude plants on LPS-induced IL-10 at RAW264.7 cell line by ELISA [Figure 1]. The result showed that GGS with the dosage more than 100 μ g/mL and 10 μ g/mL of LJF have significantly upregulated IL-10 expression at 24-h in a dose-dependent manner. However, the difference between another nine herb groups and the LPS-induced group was not statistically significant.

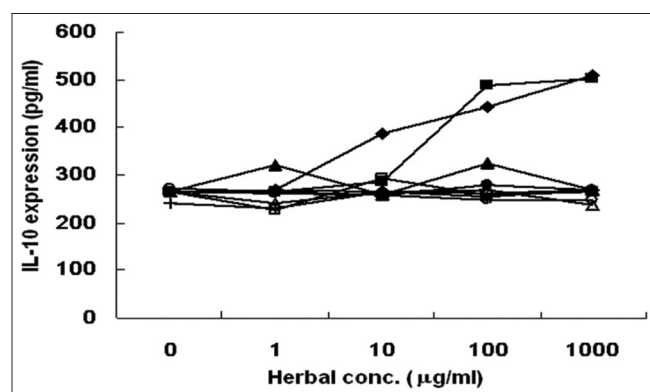


Figure 1: The herbal effect of LPS-induced IL-10 expression on RAW264.7 cells. IL-10 was induced by LPS (10 ng/ml) at 24 h and pretreated with GGS for 1 h (■) and ten herbs on RAW264.7 cell by ELISA kit. The ten herbs include *Lonicera japonica* (◆), *Forsythia suspense* (▲), *Mentha haplocalyx* (○), *Schizonepeta tenuifolia* (●), *Glycine max* (◊), *Glycyrrhiza uralensis* (△), *Platycodon grandiflorum* (□), *Lophatherum gracile* (▼), *Arctium lappa* L. (+), and *Phragmites communis* (▽). All data were means of triplicates, and numbers in parentheses indicate the standard deviation of triplicates ($n = 6$). LPS: Lipopolysaccharide, IL-10: Interleukin-10, GGS: Gingyo-san

Effects of gingyo-san and *Lonicerae japonica* flos on pro-inflammatory cytokines and interleukin-10 mRNA expression in lipopolysaccharide-stimulated RAW264.7 cells

We compared 100 μ g/mL GGS with the same dosage of LJF in the mRNA of pro-inflammatory cytokines and IL-10 by RT-PCR [Figure 2]. The data demonstrated that GGS and LJF significantly suppressed the pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, and iNOS) and upregulated IL-10. GGS is more effective than LJF in the expression of pro-inflammatory cytokines. However, LJF obviously promoted IL-10 mRNA more than GGS. The results indicated that LJF could be a major herbal ingredient of GGS on promoting the LPS-induced IL-10. In our previous publication, HPLC was employed to examine the methanol-soluble components of the LJF preparation [25]. The principal components were identified as CGA (0.0528 mg/mL) and LUT 0.00456 mg/mL. The concentration ratio of CGA and LUT is almost 10:1.

The effect of chlorogenic acid and luteolin on lipopolysaccharide-induced interleukin-10 and interleukin-6 expression

LPS induced a massive increase of IL-10 and IL-6 by ELISA. Pretreatment of LUT significantly suppressed the LPS-induced IL-6, but had no effect on IL-10 [Figure 3b and d]. In contrast, CGA can upregulate the LPS-induced IL-10 and IL-6 in a dose-dependent manner [Figure 3a and c].

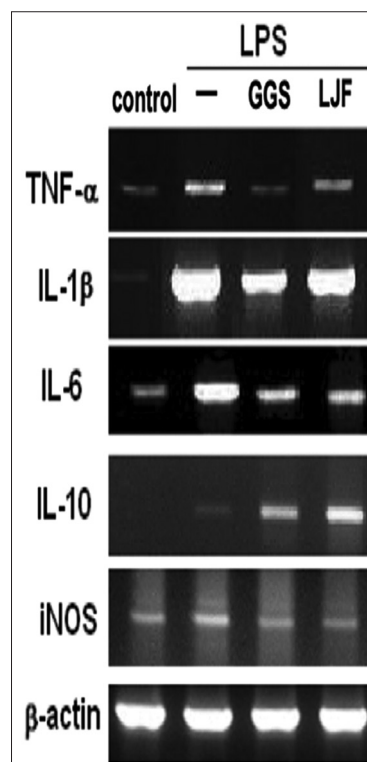


Figure 2: GGS and LJF affect LPS-induced inflammatory factor in RAW264.7 cells. RAW cells were challenged at baseline with LPS (10 ng/mL) or none ($n = 6$). Raw cells receiving LPS were pretreated with 100 μ g/mL GGS ($n = 6$) and 10 μ g/mL LJF. After 24 h, the cells were obtained and assayed. The mRNA of cells was collected, and pro-inflammatory cytokines and IL-10 were evaluated by RT-PCR. RT-PCR: Reverse transcription-polymerase chain reaction, LPS: Lipopolysaccharide, IL-10: Interleukin-10, GGS: Gingyo-san, LJF: *Lonicerae japonica* flos

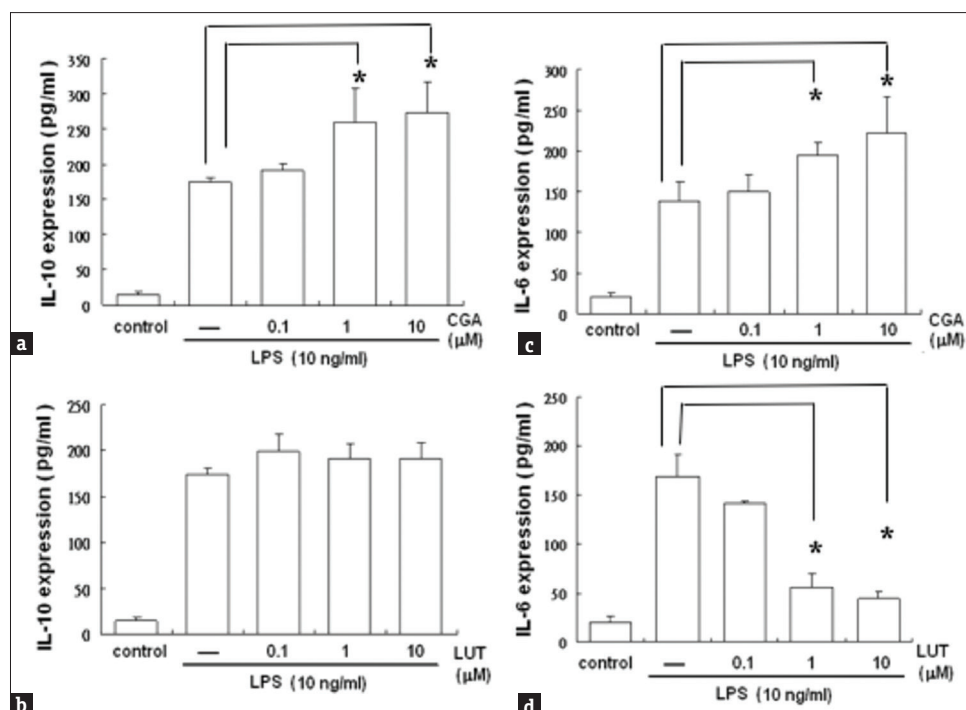


Figure 3: CGA and LUT affect LPS-induced IL-10 (a, b) and IL-6 (c, d) expression. Concentrations of IL-10 and IL-6 in the supernatant were measured in the culture medium obtained 24 h after LPS treatment (10 ng/mL) or none ($n = 6$). The cells receiving LPS were pretreated with different doses of CGA and LUT ($n = 6$). * $P < 0.05$ versus LPS treatment group. LPS: Lipopolysaccharide, IL-10: Interleukin-10, CGA: Chlorogenic acid, LUT: Luteolin

The synchronizing action of chlorogenic acid and luteolin on lipopolysaccharide-induced interleukin-10 and interleukin-6 expression

We investigated the synchronizing action of CGA (10 μM) and LUT (1 μM) on LPS-induced IL-10 and IL-6 expression by ELISA. CGA and CGA+ LUT promoted the LPS-induced IL-10 expression, whereas no effect was observed in the LUT-treated group. The levels of IL-6 in the CGS-treated group seem increased, but the difference between the LPS-treated group was not statistically significant [Figure 4a]. Respectively, IL-6 decreased significantly in LUT and CGA+ LUT groups compared with the LPS group [Figure 4b]. By integrating the two results, it can be concluded that CGA could promote LPS-induced IL-10 and IL-6 and LUT could suppress IL-6 only.

The synchronizing action of chlorogenic acid and luteolin on lipopolysaccharide-induced nuclear factor- κB , SP-1, C/EBP β , and δ activation

The expression of Sp1 and C/EBP β and δ , in nuclear extracts prepared from RAW264.7 cells treated with LPS for 6 h, was investigated by using immunoblot analysis. No difference was observed in Sp1 expression between control and LPS-treated cells [Figure 5a]. CGA at 1 and 10 μM enhanced LPS-induced Sp1 expression, but the effect of LUT on Sp1 expression was not different between CGA-treated cells [Figure 5a]. Increase of C/EBP β and δ expression was observed in cells treated with LPS for 6 h [Figure 5a]. CGA at 1 and 10 μM enhanced LPS-induced C/EBP β and δ expression but LUT inhibited LPS-induced C/EBP β and δ expression. The synchronizing action of CGA and LUT on C/EBP β and δ expression in LPS-treated cells demonstrated that

LUT reduced CGA and LPS-enhanced C/EBP β and δ expression in a dose-dependent manner [Figure 5a].

We also investigated the synchronizing action of CGA and LUT on LPS-induced NF- κB activation. Our data showed that LPS significantly induced NF- κB activation and CGA enhanced LPS-induced NF- κB activation [Figure 5b]. However, LUT has an inhibitory effect on LPS-induced and CGA-enhanced NF- κB activation in a dose-dependent manner [Figure 5a].

The synchronizing action of chlorogenic acid and luteolin on lipopolysaccharide-induced I κB pathway and mitogen-activated protein kinase pathway

For understanding the mechanism of the adaptive action of CGA and LUT, we studied the I κB and MAPK pathways that could affect NF- κB , SP-1, C/EBP β , and δ activation. CGA enhanced LPS-induced phosphorylation of I κB , ERK1/2, p38, and JNK in a dose-dependent manner; however, LUT reduced the phosphorylation of I κB , ERK1/2, p38, and JNK in a dose-dependent manner [Figure 6a]. In the adaptive action of CGA and LUT, LUT could abolish the LPS-induced or CGA-enhanced phosphorylation of I κB and p38. LUT did not influence the LPS-induced and CGA-enhanced phosphorylation of ERK1/2 and JNK [Figure 6b].

DISCUSSION

In this study, we analyzed the immunomodulatory effect of GGS and its components which enhanced LPS-induced IL-10 expression. In the event, *L. japonica*, a component of GGS, could induce the expression of IL-10. In addition, we found that CGA and LUT, the ingredients of *L. japonica*, have an adaptive effect on the increased LPS-induced IL-10 expression and the

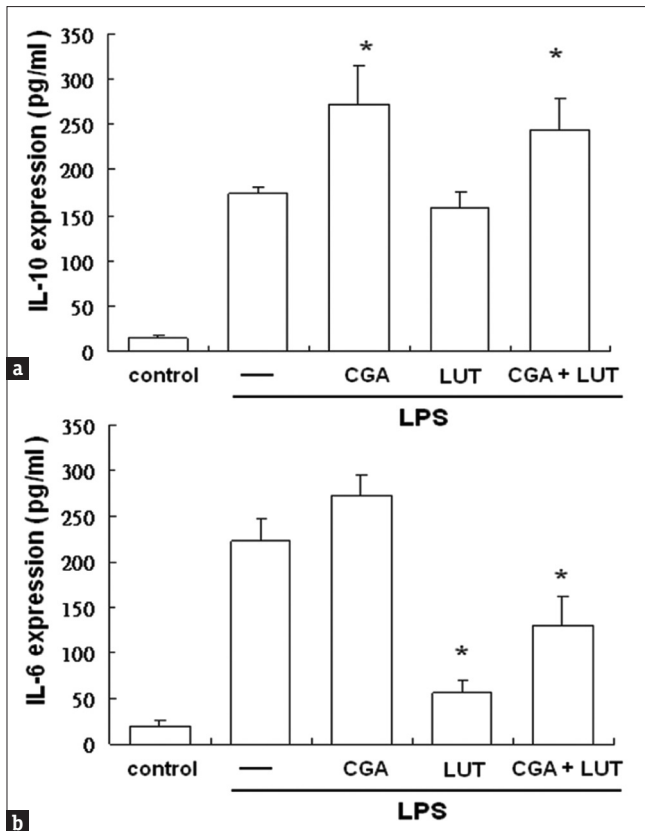


Figure 4: The synchronizing action of CGA and LUT on LPS-induced IL-10 (a) and IL-6 (b) expression. Concentrations of IL-10 and IL-6 in the supernatant were measured in the culture medium obtained 24 h after LPS treatment (10 ng/ml) or none ($n = 6$). The cells receiving LPS were pretreated with 10 μ M CGA and/or 1 μ M LUT ($n = 6$). Mean \pm SEM. * $P < 0.05$ versus LPS treatment group. LPS: Lipopolysaccharide, IL-10: Interleukin-10, CGA: Chlorogenic acid, LUT: Luteolin, SEM: Standard error of mean

decreased LPS-induced IL-6 expression. We also demonstrated that LUT could abolish the LPS-induced or CGA-enhanced phosphorylation of I κ B, ERK1/2, p38, and JNK. As a consequence, LUT has inhibitory effect on LPS-induced and CGA-enhanced NF- κ B and C/EBP β and δ activation.

LJF, also called Jinyinhua, is a widely used herb prescribed in many Chinese formulas. It has latent heat-clearing, antipyretic, detoxicant, and anti-inflammatory actions [27]. LJF not only significantly promotes blood neutrophil activity but also increases the neutrophil phagocytosis at its proper concentrations [28]. Some studies suggested that the aqueous extract of LJF flower may function as a therapeutic agent for inflammatory disease through a selective regulation of NF- κ B activation [29]. In a previous study, we also demonstrated that GGS, containing *L. japonica*, enhances LPS-induced IL-10 expression and suppresses IL-6 expression [24]. In this study, we showed tha LJF may play a major role in the inhibitory effect of TNF- α , IL-6, iNOS and in the enhancement of IL-10.

It has been reported from *in vivo* and *in vitro* experiments that CGA mostly presents antioxidant and anticarcinogenic activities [22,30]. Some reports demonstrated that CGA inhibited the peripheral synthesis/release of inflammatory

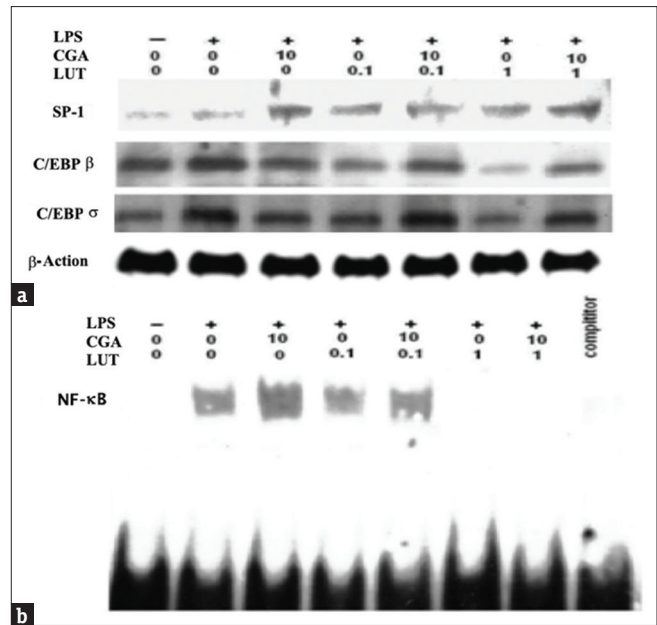


Figure 5: The synchronizing action of CGA and LUT on LPS-induced NF- κ B, SP-1, C/EBP β , and C/EBP δ activation. RAW264.7 cells were pretreated with different doses of CGA or LUT 6h before the LPS induction and the nuclear extracts were prepared and subjected to Western blotting to detect SP-1, C/EBP β , and C/EBP δ activity (a), and NF- κ B activity by EMSA (b). LPS: Lipopolysaccharide, CGA: Chlorogenic acid, LUT: Luteolin, EMSA: Electrophoretic mobility shift assay, NF- κ B: Nuclear factor- κ B

mediators, such as TNF- α and NO, but it did not inhibit the febrile response induced by LPS [31]. On the other hand, CGA has proven to augment the inducible cytokine messages, i.e., IL-10, IL-13, IL-6, TNF- α , and IFN- γ in mast cells [23]. Another compound, LUT, has been reported to decrease the production of pro-inflammatory mediators in LPS-stimulated macrophages, fibroblasts, and intestinal epithelial cells [12,14,32], and it also suppressed LPS-stimulated TNF- α and IL-6 in a murine macrophage cell line [12]. We identified two compounds (CGA and LUT) from LJF. We demonstrated that CGA enhanced LPS-induced IL-10 and IL-6, and LUT inhibited IL-6, but did not influence the expression of IL-10. Our results also showed that the adaptive action of CGA and LUT inhibited LPS-induced IL-6 and enhanced LPS-induced IL-10. Those results of the adaptive action of CGA and LUT may explain the effect of LJF on the increased LPS-induced IL-10 expression and the decreased LPS-induced IL-6 expression.

A previous study reported that the transcription factors Sp1 and C/EBP β and δ are critical for LPS-induced expression of IL-10 in mouse macrophages, and the increase in protein and DNA binding of C/EBP β and δ could activate IL-10 gene expression [10]. On the other hand, NF- κ B is an important response element located upstream of the IL-6 transcriptional initiation site, and the inhibition of NF- κ B led to a remarkable downregulation of IL-6 gene and protein expression [33]. Those transcription factors are regulated by NF- κ B and MAPK signal pathways, including ERK, JNK, and p38 [10,33]. Our data show that CGA enhanced the LPS-induction of the transcription factor Sp1, C/EBP β and δ . Consequently, the expression of IL-10 and IL-6 increased. LUT suppressed the

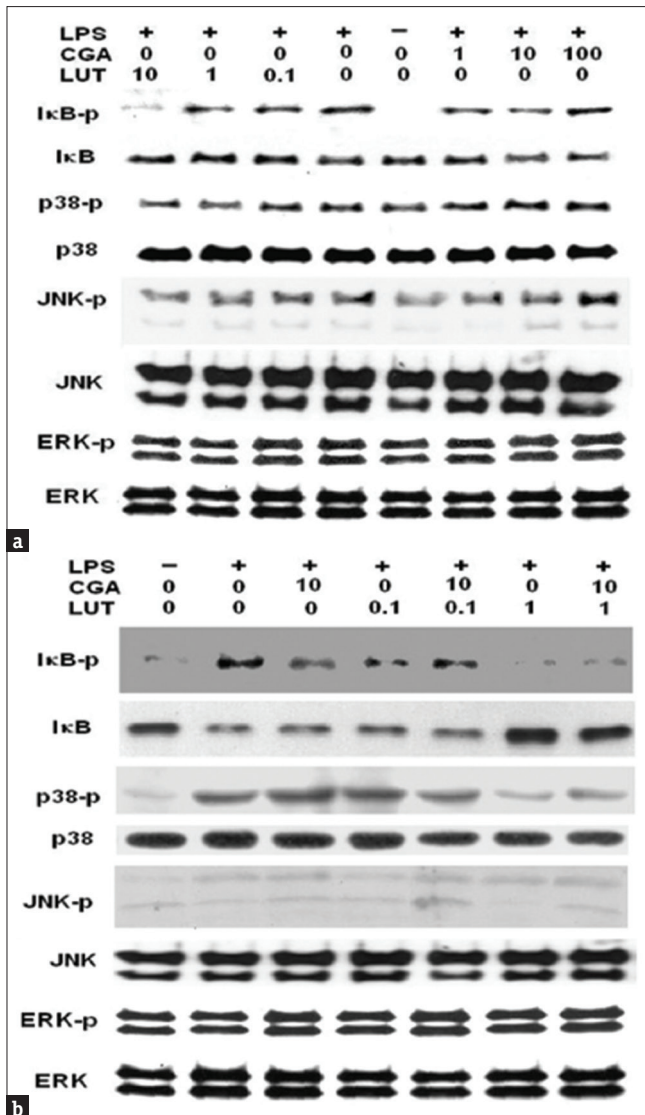


Figure 6: The synchronizing action of CGA and LUT on LPS-induced IκB, ERK1/2, p38, or JNK activation. Cells were pretreated with different doses of CGA or LUT respectively (a), and simultaneously (b) 6h before the LPS induction, and then whole cell lysates were prepared and subjected to Western blotting using antibodies specific to the phosphorylated form of IκB, ERK1/2, p38, JNK, or for IκB, ERK2, p38, or JNK as described under methods section. LPS: Lipopolysaccharide, CGA: Chlorogenic acid, LUT: Luteolin, NF-κB: Nuclear factor-κB

LPS-induced NF-κB binding ability and reduced the expression of IL-10. LUT did not influence the transcription factors Sp1 and C/EBPβ and δ. We also demonstrated the adaptive action of CGA and LUT on the suppression of the phosphorylation of IκB and p38, but not of ERK1/2 and JNK.

CONCLUSION

As illustrated in Figure 7, our present results indicate that CGA enhanced the LPS-induced expression of IL-10 and IL-6, and increased NF-κB, Sp1, C/EBPβ and δ. The effect of CGA is interfered with Lut by suppressing the phosphorylation of IκB and p38, and NF-κB activity. In the event, IL-6 was suppressed and IL-10 was not influenced.

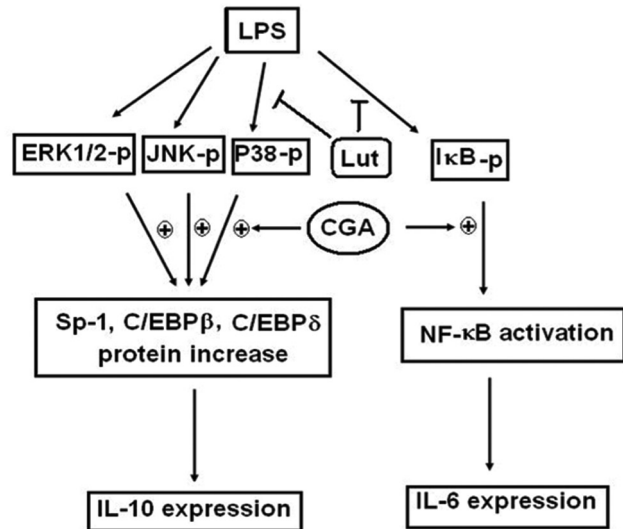


Figure 7: Schematic of adaptive immunoregulation of LUT and chlorogenic acid in LPS-induced IL-10 expression. CGA enhanced LPS-induced expression of IL-10 and IL-6 and increased the expression of NF-κB, Sp1, C/EBPβ, and δ. The effect of CGA was interfered with Lut by suppressing the phosphorylation of IκB and p38 and downregulated NF-κB activity. In the event, IL-6 was suppressed and IL-10 was not influenced. LPS: Lipopolysaccharide, CGA: Chlorogenic acid, LUT: Luteolin, NF-κB: Nuclear factor-κB, IL-10: Interleukin-10

Financial support and sponsorship

This work was supported by grants NSC 98-2320-B-303-002 from the National Science Council and DTCRD 98(2)-12 from the Buddhist Dalin Tzu Chi General Hospital, Taiwan.

Conflict of interest

There are no conflicts of interest.

REFERENCES

- Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989;170:2081-95.
- Mosmann TR, Schumacher JH, Street NF, Budd R, O'Garra A, Fong TA, et al. Diversity of cytokine synthesis and function of mouse CD4+ T cells. *Immunol Rev* 1991;123:209-29.
- de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991;174:1209-20.
- Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993;150:353-60.
- Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. *Annu Rev Immunol* 1993;11:165-90.
- Cohen SB, Crawley JB, Kahan MC, Feldmann M, Foxwell BM. Interleukin-10 rescues T cells from apoptotic cell death: Association with an upregulation of Bcl-2. *Immunology* 1997;92:1-5.
- Conti P, Kempuraj D, Kandere K, Di Gioacchino M, Barbacane RC, Castellani ML, et al. IL-10, an inflammatory/inhibitory cytokine, but not always. *Immunol Lett* 2003;86:123-9.
- Brightbill HD, Plevy SE, Modlin RL, Smale ST. A prominent role for Sp1 during lipopolysaccharide-mediated induction of the IL-10 promoter in macrophages. *J Immunol* 2000;164:1940-51.
- Ma W, Lim W, Gee K, Aucoin S, Nandan D, Kozlowski M, et al.

- The p38 mitogen-activated kinase pathway regulates the human interleukin-10 promoter via the activation of Sp1 transcription factor in lipopolysaccharide-stimulated human macrophages. *J Biol Chem* 2001;276:13664-74.
10. Liu YW, Chen CC, Tseng HP, Chang WC. Lipopolysaccharide-induced transcriptional activation of interleukin-10 is mediated by MAPK- and NF-kappaB-induced CCAAT/enhancer-binding protein delta in mouse macrophages. *Cell Signal* 2006;18:1492-500.
 11. Choi CW, Jung HA, Kang SS, Choi JS. Antioxidant constituents and a new triterpenoid glycoside from flos *Lonicerae*. *Arch Pharm Res* 2007;30:1-7.
 12. Xagorari A, Papapetropoulos A, Mauromatis A, Economou M, Fotsis T, Roussos C. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. *J Pharmacol Exp Ther* 2001;296:181-7.
 13. Chen CY, Peng WH, Tsai KD, Hsu SL. Luteolin suppresses inflammation-associated gene expression by blocking NF-kappaB and AP-1 activation pathway in mouse alveolar macrophages. *Life Sci* 2007;81:1602-14.
 14. Kim JS, Jobin C. The flavonoid luteolin prevents lipopolysaccharide-induced NF-kappaB signalling and gene expression by blocking IkappaB kinase activity in intestinal epithelial cells and bone-marrow derived dendritic cells. *Immunology* 2005;115:375-87.
 15. Clifford MN, Jaganath IB, Ludwig IA, Crozier A. Chlorogenic acids and the acyl-quinic acids: Discovery, biosynthesis, bioavailability and bioactivity. *Nat Prod Rep* 2017;34:1391-421.
 16. Wu HZ, Luo J, Yin YX, Wei Q. Effects of chlorogenic acid, an active compound activating calcineurin, purified from flos *Lonicerae* on macrophage. *Acta Pharmacol Sin* 2004;25:1685-9.
 17. Tsuchiya T, Suzuki O, Igarashi K. Protective effects of chlorogenic acid on paraquat-induced oxidative stress in rats. *Biosci Biotechnol Biochem* 1996;60:765-8.
 18. Natella F, Nardini M, Giannetti I, Dattilo C, Scaccini C. Coffee drinking influences plasma antioxidant capacity in humans. *J Agric Food Chem* 2002;50:6211-6.
 19. Tanaka T, Kojima T, Kawamori T, Wang A, Suzui M, Okamoto K, et al. Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. *Carcinogenesis* 1993;14:1321-5.
 20. Tanaka T, Nishikawa A, Shima H, Sugie S, Shinoda T, Yoshimi N, et al. Inhibitory effects of chlorogenic acid, reserpine, polyprenoic acid (E-5166), or coffee on hepatocarcinogenesis in rats and hamsters. *Basic Life Sci* 1990;52:429-40.
 21. Zhou J, Xuan B, Li DX. Effects of tetrahydroberberine on ischemic and reperfused myocardium in rats. *Zhongguo Yao Li Xue Bao* 1993;14:130-3.
 22. Feng R, Lu Y, Bowman LL, Qian Y, Castranova V, Ding M. Inhibition of activator protein-1, NF-kappaB, and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. *J Biol Chem* 2005;280:27888-95.
 23. Chen S, Gong J, Liu F, Mohammed U. Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation. *Immunology* 2000;100:471-80.
 24. Yeh CC, Lin CC, Wang SD, Hung CM, Yeh MH, Liu CJ, et al. Protective and immunomodulatory effect of gingyo-san in a murine model of acute lung inflammation. *J Ethnopharmacol* 2007;111:418-26.
 25. Kao ST, Liu CJ, Yeh CC. Protective and immunomodulatory effect of flos *Lonicerae Japonicae* by augmenting IL-10 expression in a murine model of acute lung inflammation. *J Ethnopharmacol* 2015;168:108-15.
 26. Yeh CC, Kao SJ, Lin CC, Wang SD, Liu CJ, Kao ST. The immunomodulation of endotoxin-induced acute lung injury by hesperidin *in vivo* and *in vitro*. *Life Sci* 2007;80:1821-31.
 27. Lan W, Zhaojun Z, Zesheng Z. Characterization of antioxidant activity of extracts from flos *Lonicerae*. *Drug Dev Ind Pharm* 2007;33:841-7.
 28. Hu S, Cai W, Ye J, Qian Z, Sun Z. Influence of medicinal herbs on phagocytosis by bovine neutrophils. *Zentralbl Veterinarmed A* 1992;39:593-9.
 29. Lee JH, Ko WS, Kim YH, Kang HS, Kim HD, Choi BT. Anti-inflammatory effect of the aqueous extract from *Lonicera japonica* flower is related to inhibition of NF-kappaB activation through reducing I-kappaBalpha degradation in rat liver. *Int J Mol Med* 2001;7:79-83.
 30. Niggeweg R, Michael AJ, Martin C. Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nat Biotechnol* 2004;22:746-54.
 31. dos Santos MD, Almeida MC, Lopes NP, de Souza GE. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biol Pharm Bull* 2006;29:2236-40.
 32. Kim SH, Shin KJ, Kim D, Kim YH, Han MS, Lee TG, et al. Luteolin inhibits the nuclear factor-kappa B transcriptional activity in rat-1 fibroblasts. *Biochem Pharmacol* 2003;66:955-63.
 33. Squarize CH, Castilho RM, Sriuranpong V, Pinto DS Jr., Gutkind JS. Molecular cross-talk between the NFkappaB and STAT3 signaling pathways in head and neck squamous cell carcinoma. *Neoplasia* 2006;8:733-46.